Genetic Diversity and Oil Quality of *Guizotia* Cass. (Asteraceae)

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Doctoral Thesis
Swedish University of Agricultural Sciences
Alnarp 2008

Acta Universitatis agriculturae Sueciae
2008:XXX

Cover: Inflorescences of niger

ISSN 1652-6880
ISBN 978-91-86195-18-2

Tryck: SLU Service/Repro, Alnarp 2008

Abstract

Petros, Y. 2008. Genetic Diversity and Oil Quality of Guizotia Cass. (Asteraceae). Doctoral Dissertation.
ISSN 1652-6880, ISBN 978-91-86195-18-2

Genetic diversity of *G. abyssinica* (L.f) Cass., *G. scabra* (Vis.) Chiov. ssp. *scabra*; *G. scabra* (Vis.) Chiov. ssp. *schimperi* (Sch. Bip.) Baagoe; *G. villosa S*ch. Bip., *G. zavattarii* Lanza and *G. arborescens* (I. Friis) collected from Ethiopia were studied using Inter Simple Sequence Repeat (ISSR) markers. Higher genetic diversity was revealed among the individuals belonging to the same population than among the populations of the different regions. Overall, greater variation was observed between the niger populations originating from Wollo and Hararghe on the one hand and those from the rest of the regions on the other. Among the wild *Guizotia* species, *G. scabra* ssp. *schimperi* was found to be closer to *G. villosa* than to any of the wild *Guizotia* taxa. Likewise, *G. zavattarii* and *G. arborescens* are found to be more closely related to each other than to the rest of the wild taxa. Based on the ISSR results, revision of the previous classification that placed *G. scabra* ssp. s*chimperi* as a sub species of *G. scabra* was suggested.

Both the field evaluation of agronomic characters as well as the ISSR analysis revealed variation among the niger populations grown in different regions of the country. Based on the agronomic characters, it was observed that the niger populations obtained from Wollo and Hararghe are of the early maturing types while the accessions originating from the rest of the regions are mostly of the late maturing types. The early maturing and the late maturing niger types differ in many of their agronomic characters notable among which are days to flower initiation, days to 50% flowering, plant height and seed size.

A niger breeding experiment was undertaken in an attempt to elevate the oleic acid content in the seed oil. The objective to increase the oleic acid content in niger seed oil from what it is today, approximately 5-11% in the "wild type" niger of Ethiopian origin to over 80% in the strains improved for oleic acid content has been achieved. The increase in the oleic acid content of the seeds has been gradual. Niger strains that are true breeding for high oleic acid content of over 80% were obtained after three rounds of selection and breeding.

Keywords:Africa, Ethiopia, Fatty acid, Genetic diversity, Guizotia, ISSR, niger, oleic acid

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Dedication

To A chatholic nun, the late Sister Helen
My uncle, the late Mikael Bartholome and
My mother, the late Yowanna Bartholome.

"Take heed that ye despise not one of these little ones; for their angels do always behold the face of my father which is in heaven" Mat 18:10

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Petros, Y., Merker, A. & Zeleke, H. 2007. Analysis of genetic diversity of *Guizotia abyssinica* from Ethiopia using inter simple sequence repeat markers. *Hereditas* 144, 18-24.

II Petros, Y., Merker, A. & Zeleke, H. 2008. Analysis of genetic diversity and relationships of wild *Guizotia* species from Ethiopia using ISSR markers. *Genetic Resources and Crop Evolution* 55, 451-458.

III Petros, Y., Zeleke, H. & Merker, A. Quantitative trait variation of *Guizotia abyssinica* (L.f) Cass. Collected from Ethiopia. Submitted

IV Petros, Y., Carlsson, A.S., Stymne, S., Zeleke, H., Fält, A-S. & Merker, A. Developing high oleic acid *Guizotia abyssinica* (L.f). Cass. by plant breeding. *Plant Breeding* Accepted pending revision

1 Introduction

1.1 Background History

The genus *Guizotia* belongs to the family Asteraceae (Compositae), tribe Heliantheae, sub tribe Coreopsidinae. The taxonomic revision of the genus was done by Baagoe (1974) who reduced the number of species to six, five of which grow in Ethiopia. These are *G. abyssinica* (L.f) Cass., *G. scabra* (Vis) Chiov. ssp. *scabra*; *G. scabra* (Vis) Chiov. ssp. *schimperi* (Sch. Bip.) Baagoe; *G. villosa* Sch. Bip., *G. zavattarii* Lanza; *G. arborescens* (I. Friis) and *G. jacksoni* (S. Moore) J. Baagoe.

G. abyssinica (niger in English), being the only cultivated member of the taxon is the most important economically. Niger is also the only species that is found outside Africa (Dagne and Heneen, 1992). To date, there is an unresolved controversy regarding the taxonomic position of *G. scabra* ssp. *schimperi* vis-à-vis *G. abyssinica*. The argument is whether *G. scabra* ssp. *schimperi* is more closely related to *G. abyssinica* than to *G. scabra* ssp. *scabra* as classified by Baagoe (1974). To this effect the bulk of the evidence points to the close similarity of *G. scabra* ssp. *schimperi* to *G. abyssinica* than to *G. scabra* ssp. *scabra* and the suggestion that *G. scabra* ssp. *schimperi* be given a specific status on its own (Murthy et al., 1993; Dagne, 1994a; Geleta et al., 2007a; Petros et al., 2008). Even after the publication of the revision work on the taxonomy of the genus by Baagoe in 1974, some authors continued to refer to the two sub species as *G. scabra* and G. *schimperi* (Murthy et al., 1995). The most recent workers supporting the independent specific status of *G. scabra* ssp. *schimperi* base their argument on molecular similarity, cytology, crossability and phytogeography as well as on morphological similarities (Dagne, 1994a; Murthy et al., 1993; Murthy et al., 1995; Geleta et al., 2007a; Petros et al., 2008). The controversy regarding the taxonomic status of *G. scabra* ssp. *schimperi* is still continuing and will probably continue until further revision of the taxon that takes into account the cytology, molecular diversity as well as morphological features is undertaken.

1.2 Botanical Description

Guizotia are dicotyledonous plants. There are both herbaceous and woody members as well as annual and perennial ones. *G. abyssinica* is easily distinguished from the other members of the taxon by its large achenes and large head size as well as ovate outer phyllaries (Dagne, 1994b). *G. scabra* contains two sub species; *G. scabra* ssp. *scabra* and *G. scabra* ssp. *schimperi*, easily distinguishable from each other by their growth habit. *G. scabra* ssp. *scabra* is a perennial plant that is found usually in the wild as a component of the natural vegetation while *G. scabra* ssp. *schimperi* is annual and a common weed in the cultivation of crop plants. They are also distinguished by the number of their ray and disc florets. In *G. scabra* ssp. *scabra,* the number of ray florets and disc florets are 8-16 and >50 respectively, while *G. scabra* ssp. *schimperi* is reported to have lower number of florets (Murthy et al., 1993; Dagne, 1994b). *G. arborescens* is a rare perennial shrub. It is distinguished from the other *Guizotia* species by its woody habit and petiolated leaves. *G. villosa* is an annual plant with profuse branching, and with linear outer involucral leaves (Hiremath and Murthy, 1988; Dagne, 1994b). *G. zavattarii* is a perennial taxon distinguished from the other *Guizotia* taxa by its pandurate leaves and oblong outer phyllaries (Dagne, 1994b). *G. jacksoni* is a perennial plant, the only member of the genus not found in Ethiopia. Its creeping growth habit and relatively large achenes which rate second among the *Guizotia* only to that of *G. abyssinica* are its distinguishing features (Dagne, 1994b).

1.3 Geographical Distribution

All *Guizotia* species grow in tropical Africa especially in East Africa with a greater concentration in Ethiopia (Hiremath and Murthy, 1988). In fact, five out of the six *Guizotia* species described are found in Ethiopia. *G. villosa* is endemic to northern Ethiopia (Dagne, 1994b), and two *Guizotia* species that are not yet described and known only by the name of their localities, 'Ketcha' and 'Chelellu' are reported only from Ethiopia (Dagne, 2001). *G. abyssinica* is cultivated in Ethiopia and the Indian sub continent as a source of edible oilseed (Murthy et al., 1993). Its cultivation in Ethiopia is mainly in Gojam, Gonder, Shewa and Wellega (Genet and Belete, 2000; Getinet and Sharma, 1996), though it is also grown to a lesser extent in Wollo, Harrarghe, Arsi, Bale and Jimma (Getinet and Sharma, 1996). In Hararghe and Wollo it is sown as a hedge around teff fields. Although the utility of

this practice is not understood it is, however, indicated by Getinet and Sharma (1996) that it may serve to protect the crop it is surrounding from being eaten by animals as it is reported that cattle do not consume niger.

Although all the wild *Guizotia* species grow in tropical Africa, the extent of their distribution varies greatly. Some of the species such as *G. villosa, G. arborescence, G. zavattarii* and *G. jacksoni* are restricted in their distribution, while others like *G. scabra* ssp *scabra*, and *G. scabra* ssp *schimperi* cover a relatively wide area in east Africa with a greater concentration in Ethiopia. *G. arborescence* is a component of the natural vegetation in the Imatong mountains of Sudan and Uganda and southern Ethiopia (Friis, 1971; Baagoe, 1974; Hiremath and Murthy, 1988). *G. zavattarii* is endemic to mount Mega in southern Ethiopia and the Huri hills in northern Kenya (Dagne, 1994b; Baagoe, 1974; Hiremath and Murthy, 1988). The distribution of *G. scabra* ssp *scabra* covers a wide range extending from east Africa to Nigeria with a distributional gap in the rain forest of Congo, while *G. scabra* ssp *schimperi* is a common weed of cultivated crops and widely distributed in Ethiopia (Hiremath and Murthy, 1988; Dagne, 1994b). *G. villosa* is endemic to the northern part of Ethiopia and *G. jacksoni* is endemic to Mt. Kenya, Aberdares and Mt. Elgon in Uganda and Kenya (Dagne, 1994b).

1.4 Cytology

Cytological studies were undertaken by several workers on the members of the genus. These works helped to shade some light on the karyotypes and chromosomes of the *Guizotia* species (Hiremath and Murthy, 1992; Murthy et al., 1993; Dagne and Heneen, 1992; Dagne 1994a, b, 1995, 2001; Dagne et al., 2000). As a result of the cytological work so far done, it has now been established that all members of the genus are diploid plants with a chromosome number of 2n=30 (Hiremath and Murthy, 1992, 1995; Dagne and Heneen, 1992; Dagne, 1995, 2001). Dagne (2001) described the chromosome morphology of two new *Guizotia* species from Ethiopia and showed that their chromosome number is indeed 2n=30 as in the other known *Guizotia* species (Dagne, 1995). Interspecific hybridization works among the *Guizotia* species elucidated the genetic relationships and relatedness among members of the taxa (Dagne, 1994a; Hiremath and Murthy, 1988; Murthy et al., 1993). Dagne and Heneen (1992) reported four pairs of satellite chromosomes and C-banding for *G. abyssinica*. B-chromosomes were observed in some samples of *G. scabra* ssp. *scabra* from

Ethiopia that was not observed among populations of the species elsewhere (Hiremath and Murthy, 1986; Dagne, 1994c).

1.5 Domestication of niger

The progenitor of niger is believed to be *G. scabra* ssp. *schimperi* as these two species share many common features both in morphology and cytology as well as ecological habitat. Niger is thought to have originated in Ethiopia through selection and cultivation of plants with large sized achenes of *G. scabra* ssp. *schimperi* prior to 3000 BC and introduced into India via trade routes before the Christian era (Hiremath and Murthy, 1988). One convincing evidence that Ethiopia may probably be the center of origin of niger is the fact that all of the wild *Guizotia* species excepting one (*G. jacksoni*) are found in Ethiopia and none in India (Murthy et al., 1993). The other evidence is the occurrence of wild *G. abyssinica* populations in areas not under cultivation in Ethiopia (Getinet and Sharma, 1996).

Of the known *Guizotia* taxa, *G. scabra* ssp. *schimperi* is the most likely candidate to be considered as the putative progenitor of niger. Supporting evidence for this assumption comes from studies of interspecific hybridization and comparison of the chromosomes of these two species (Dagne, 1994a; Murthy et al., 1993, 1995). *G. abyssinica*, *G. scabra* ssp.*schimperi* and *G. villosa* are more closely related to each other than to the other members of the genus *Guizotia* (Dagne, 1994a; Murthy et al., 1993; Murthy et al., 1995; Hiremath and Murthy, 1988,1992; Bekele et al., 2007). The assertion of their close similarity comes from the evidence of their karyotype similarity which is symmetrical unlike the other *Guizotia* species studied that have asymmetrical karyotypes (Murthy et al., 1993; Dagne, 1994a). The same conclusion is reached by Dagne (1994a) based on the extent of crossability among members of the *Guizotia* taxa. The works of Dagne (1994a) and Murthy et al. (1993) also revealed that chromosome homology is higher between *G. abyssinica* and *G. scabra* ssp. *schimperi* than between any other *Guizotia* taxa. As the extent of chromosome pairing in the F1 hybrids is widely regarded as indicative of species relatedness, *G. scabra* ssp. *schimperi* is considered to be closely related to *G. abyssinica* in this regard compared to the other wild or weedy *Guizotia* species. Murthy et al (1993) also indicated that *G. abyssinica* and *G. scabra* ssp. *schimperi* share more karyomorphological features with each other than with any of the other *Guizotia* species including the extent of bivalent formation and the degree of the fertility of the hybrids which led them to conclude that the genome of

G. abyssinica and *G. scabra* ssp. *schimperi* are basically similar and fully homologous. It is probable that the genomes of *G. abyssinica* and *G. scabra* ssp. *schimperi* became distinctly differentiated during the course of evolution by a single reciprocal translocation (Hiremath and Murthy, 1992; Murthy et al., 1993). The crossability between *G. abyssinica* and *G. scabra* ssp *schimperi* is reported to be 51.8%. They produce fully fertile hybrids the cells of which show 15 bivalent formation during the prophase of the first meiotic cell division (Hiremath and Murthy, 1995).

Hiremath and Murthy (1995) also present a different but an equally likely scenario that *G. abyssinica* and *G. scabra* ssp. *schimperi* might have evolved from a common ancestor and suggested that *G. scabra* ssp. *schimperi* be classified under *G. abyssinica* with sub specific rank while Bekele et al. (2007), based on the sequences from the internal transcribed spacers of the nuclear ribosomal DNA suggested the possibility that G.*scabra* ssp. *scabra*, *G. scabra* ssp. *schimperi* and *G. villosa* could have contributed to the evolution of the cultivated *G. abyssinica*. Geleta et al. (2007a) after RAPD and AFLP analysis of the *Guizotia* species indicated the close similarity of *G. scabra* ssp. *schimperi* to *G. abyssinica* than to *G. scabra* ssp. *scabra* and suggested that *G. scabra* ssp. *schimperi* merits a specific status on its own.

1.6 Economic Importance of niger

Niger is the only cultivated species of the genus and economically the most important because it is used for human consumption. In Ethiopia 50-60% of the edible oil requirement for domestic consumption is obtained from niger seed (Riley and Belayneh, 1989). In India, though not the main source, it is used as a source of edible oil (Hiremath and Murthy, 1993). In Ethiopia, niger seed oil is extracted through traditional extraction method that entails crushing, warming and traditional centrifugation (Getinet and Sharma, 1996). Nowadays, large oil mills located in the major cities and towns in Ethiopia extract niger seed oil. The left-behind after oil extraction is rich in protein and fiber and can be used as animal feed (Ramadan and Morsel, 2002; Kandel and Porter, 2002). In the United States, niger seed is used as food for birds especially finches. It is also used in the making of soap and as carrier of scent in perfume industry (Kandel and Porter, 2002). The higher percentage of linoleic acid gives the niger seed oil from Ethiopian origin superior quality for use in paints (Kandel and Porter, 2002). Niger meal can also be used as a relatively cheaper growth medium for *Bacillus* species responsible for the production of alkaline protease (Gessesse, 1997).

Moreover, consuming niger seed oil is beneficial from public health point of view because it contains minor quantities of substances such as tocopherols, phospholipids and sterols that provide protection against cardiovascular disorders and cancer (Ramadan and Morsel, 2002).

1.7 Agronomic considerations

Niger is a short day plant and does not flower when the day length is more than 12 hours. After the induction of flowering, however, it would continue to produce flowers even if it is subjected to longer duration of daylight (Getinet and Sharma, 1996). The Ethiopian niger requires an optimum of 18°C day time temperature and 13°C night time temperature (Getinet and Sharma, 1996).

There are three types of niger growing in Ethiopia that are differentiated based on the duration to maturity. These are the early maturing type called 'Bunigne', a late maturing type called 'Abat' and a frost resistant type called 'Mesno' niger (Getinet and Sharma, 1996; Almaw and Teklewold, 1995). In Ethiopia the sowing date differs depending on the different maturity types. The 'Abat' type is sown in mid May to early June, 'Bunigne' in July and 'Mesno' niger in September. All the three maturity groups flower when the day length is shortened and harvested in October (Bunigne), December (Abat) and February (Mesno). The optimum seeding rate varies from 5-10 kg/ha and 5-8kg/ha for the Ethiopian and the Indian niger respectively (Getinet and Sharma, 1996). Kandel (2004) reported the optimum seeding rate to be 127seeds/m2 for an early maturing type 'early bird' developed in the United states. Altitudinal variations of the growing areas of niger in Ethiopia range for the most part from 1600-2200masl (Getinet and Sharma, 1996).

In Ethiopia, niger is cultivated in rotation with cereal crops like teff. It is reported that the cereals that follow niger in cultivation perform better with little infestation by weeds (Getinet and Sharma, 1996). It is also reported that an exudate from niger inhibits the growth of monocotyledonous weeds. Niger is tolerant to water logging and often grown in marginal areas with little or no fertilizer requirements (Alemaw and Teklewold, 1995). The tolerance to water logging is due to the development of extensive aerenchyma when the plants are subjected to repeated water logging conditions (Getinet and Sharma, 1996; Petros, 1998). It is reported that

Nitrogen fertilizers generally increase vegetative growth but do not increase the seed yield of niger (Almaw and Teklewold, 1995).

Getinet and Sharma (1996) cited four improved varieties of niger, Sendafa, Fogera, Esete and Kuyu released by the Institute of Agricultural Research, Holeta Research Center. From the works of Getinet and Sharma (1996), it seemed that the variety Kuyu is a higher yielding variety both in terms of seed yield, 1060Kg/ha and oil content, 41%. It also happened that compared with the other varieties released to farmers in Ethiopia, Kuyu possesses most of the desirable qualities like earliness and short stature. If higher seed yield is also accompanied by higher oil content, it seems promising that agronomists work to increase the seed yield or select and breed for high oil content, as the ultimate breeding objective of niger is to increase the amount of oil per unit area (Teklewold and Wakjira, 2004). The future research of niger should make the development of hybrid varieties with desirable qualities its priority, provided varied heterotic strains are found among the country's germplasm (Getinet and Sharma, 1996).

1.8 Oil content of Niger

Niger is an important oilseed crop in Ethiopia where it provides about 50-60% of the oil for domestic consumption (Riley and Belayneh, 1989). It is also used as an oilseed crop in India where it provides about 3% of the edible oil requirement of the country (Getinet and Sharma, 1996). The oil content of niger is reported by Dagne and Johnsson (1997) as ranging from 42-44% of the seed weight. Dutta et al. (1994) reported 29-39% oil in niger seed collected from different regions of Ethiopia while Almaw and Teklewold (1995) reported the oil content as ranging from 39.8-46.9%. Seegeler (1983) and Kandel and Porter (2002) reported oil contents of 30-50% and 30-35% respectively. Other than being very important for their nutritional value, oilseeds have considerable importance for industrial and pharmaceutical purposes (Ramadan and Morsel, 2003a).

Niger seed oil contains neutral lipids as well as polar lipids and sterols (Ramadan and Morsel, 2003a, 2003b, 2003c). The presence of polar lipids like glycolipids and phospholipids in appreciable amounts in niger seeds is reported by Ramadan and Morsel (2003a, b). Neutral lipids are the dominant class of lipids in niger seed oil amounting to 93% and 97% of the total lipids depending on the methods of extraction, followed by glycolipids at about 4.9% and phospholipids 0.6% (Ramadan and Morsel, 2002, 2003b).

Triacylglycerols are the major components of the neutral lipid class making up 89.7-91.9% of the neutral lipids in niger seed oil (Ramadan and Morsel, 2002). There are four major fatty acids in niger seed oil. These include two main unsaturated fatty acids, linoleic acid (18:2) and oleic acid (18:1) and two major saturated fatty acids, palmitic acid (16:0) and stearic acid (18:0). The abundance of these major fatty acids as well as some minor fatty acids reported to be present in small or trace amounts varies greatly from one sample to the other. Dagne and Johnsson (1997) reported the abundance of linoleic acid as ranging from 65.7-68%, oleic acid, 5.4-7.5%, palmitic acid 9.6-10% and stearic acid 7.6-8.1% while Ramadan and Morsel 2003c reported 63%, 11%, 17% and 7% for the percentage composition of linoleic, oleic, palmitic and stearic acids respectively in niger seed oil.

Though the variation in the oil content of niger cannot be accounted for by the location or the climatic condition of the area as indicated by Dutta et al. (1994), variation in the fatty acid profile, however, can be attributed to several factors such as the area of origin of the material, the climatic condition of the area and more importantly genetic variation. In this regard, niger samples from India are reported to yield relatively high oleic acid. It is also reported that higher temperatures particularly during the period of flowering and fruit set would favour the production of increased oleic acid levels in sunflower (Rondanini et al., 2003). Thus higher temperatures during the reproductive phase of the plant would tilt the balance of the oleic /linoleic ratio towards the production of higher levels of oleic acid in the seed. The increase in the oleic acid content of the seed in this case is compensated for by a relatively reduced level of linoleic acid. In general cooler temperatures favour the production of linoleic acid where it is reported to rise as high as 85% in niger while higher temperatures have the reverse effect on the production of the acid. Linoleic acid is the predominant fatty acid among the neutral lipids as well as the glycolipid fraction of the oil (Ramadan and Morsel, 2003a, b).

The major phospholipid bound fatty acids in niger are linoleic, palmitic and oleic acid (Ramadan and Morsel, 2003b). Among the polar lipids in niger, phospholipids contain relatively high proportions of palmitic acid, the percentage increase of which is compensated by a relative reduction in the proportion of linoleic acid (Dutta et al., 1994). Dutta et al (1994) also reported -tocopherol as the predominant tocopherol in niger seed oil as well as sterols of which -sitosterol predominates occurring at 2000μg/g oil. The high tocopherol content of niger seed oil is of great dietary significance because tocopherols act as antioxidants particularly in light of the high

proportion of the poly unsaturated fatty acid (18:2) which renders the oil prone to oxidative deterioration. The oxidative stability of niger seed oil containing higher percentage of linoleic acid even than those reported for sunflower and safflower (Gecgel et al., 2007; Martinez et al., 1993), can be attributed to the tocopherol content that serves as antioxidant in the oil. It is the crude niger seed oil that is traditionally consumed in Ethiopia (Getinet and Sharma, 1996). This natural oil contains minor constituents that would be removed from the oil at several stages in the refining process. These minor constituents such as tocopherols, and phospholipids have antioxidant activity (Ramadan and Morsel, 2002). Phytosterols are known to be hypocholestrolemic and the consumption of oils rich in these antioxidants is believed to provide protection against cancer, cerebrovascular and cardiovascular diseases (Ramadan and Morsel, 2002).

Teklewold and Wakjira (2004) studied the pattern and rate of seed dry weight and oil accumulation in two improved varieties, Fogera and Kuyu. They observed that the critical duration for the accumulation of oil in niger seed was between 15 to 35 days after anthesis initiation where the oil accumulation increases from 11.68 to 40.6%. They pointed out that the amount of oil would tend to slightly decrease until harvesting date thereby leading to a reduction in the oil content and the seed dry weight. Thus, timely harvest is recommended to maximize the produce of niger seed oil.

1.9 Genetic Diversity

Molecular studies revealing the genetic diversity of niger are indeed scanty. The few genetic diversity studies so far done using DNA markers reveal that the genetic variability of the *Guizotia* species is high both within and among populations (Petros, 2007, 2008). Random Amplified Polymorphic DNA (RAPD) (Geleta et al., 2007b) and Amplified Fragment Length Polymorphism (AFLP) (Geleta et al., 2008) also revealed high genetic diversity for *G. abyssinica* populations from Ethiopia. The genetic variability as a function of percent polymorphic loci as well as the Shannon-Weaver diversity indices was reported to be higher for *G. abyssinica* than the other wild *Guizotia* species (Petros et al., 2007, 2008). The within population genetic diversity among all the *Guizotia* populations is reported to be higher than the among population genetic diversity (Geleta et al., 2007b, c, 2008; Petros et al., 2007, 2008). Geleta et al (2007d) made a molecular phylogenetic analysis of the *Guizotia* species based on chloroplast DNA

sequences and suggested the transfer of the genus from its present sub tribe Coreopsidinae to the sub tribe Milleriinae. They also indicated that the perennial forms within the genus might have been the first to evolve.

1.10 Breeding and Biotechnology

Niger is completely out crossing and highly self incompatible. The self incompatibility of niger is homomorphic and of the sporophytic type (Prasad, 1990; Almaw and Teklewold 1995). In self incompatible forms of niger the pollen fails to germinate normally but instead twists and coils over the stigmatic papillae (Prasad, 1990). *Guizotia* species can interbreed normally in nature producing F1 hybrids and even more so when they happen to grow in close proximity with each other (Dagne, 1994a). The F1 interspecific hybrids show quantitative characters that are intermediate between the parents but express the qualitative characters of the male parent (Murthy et al., 1993). Performance of interspecific hybrids is a valuable indicator of the feasibility of transferring valuable agronomic traits from the wild or weedy species to the cultivated one (Murthy et al., 1993).

Adda et al (1994a) produced diploid plants from anther cultures of niger that showed significant variation with regards to some traits of agronomic importance. These were self compatible short plants with large head size. This finding is all the more important as self incompatibility is the main problem on the way of the realization of the full reproductive potential of niger (Adda et al., 1994b). Reduced height coupled with high yield is in fact the most desirable character if the production of niger is to be amenable to mechanization such as the use of combine harvester. Adda et al. (1994b) also developed a protocol for the regeneration of niger from the cotyledons through somatic embryogenesis.

The single most important objective to be considered when undertaking niger improvement programs is to increase the amount of oil per unit area of land (Teklewold and Wakjira, 2004). This objective embodies two facets each of which lead to the same ultimate goal but can be carried out in two different directions. These are either increasing the seed yield by breeding for high seed yielding varieties or developing varieties of niger with high oil content. As it stands today, niger is a low yielding crop whose cultivation is plagued by a number of critical drawbacks. Among these factors that severely limit the realization of the full production potential of the crop are indeterminate growth habit leading to non-synchronous maturity ultimately leading to seed shattering and a severe loss of yield and self incompatibility

that, among other things, contributes to the reduction in seed yield. The height of the plant is also an important agronomic attribute that needs thoughtful consideration because at its present tall stature it is very unlikely that mechanized farming can be practiced in the production of niger. In order to exploit the full potential of the crop, single headed dwarf types need to be developed (Getinet and Sharma, 1996). There is, however, ample opportunity for researchers in the area to develop niger varieties with the desired agronomic qualities because the Ethiopian niger is inherently diverse with a wealth of genetic variability. The Ethiopian germplasm collection contains the three known maturity groups. As there is a host of strikingly contrasting features particularly between the early maturing 'Bunigne' and the late maturing 'Abat' niger, there appears to be ample resources present for the improvement of the crop by selection and breeding for desirable agronomic qualities from among the niger populations growing in the country. The early and late maturing types of niger differ with respect to a number of important characters such as heads per plant, seeds per head, seed weight, plant height, days to maturity and number of primary branches. The niger populations of Ethiopia, thus, present rich genetic variability to be utilized for the development of niger varieties with ideal agronomic characters.

The other direction that niger improvement program could follow but which also aid to achieve the same ultimate goal is to breed for increased oil content in niger seed. To date, there are few works done on the oil content of niger seeds from Ethiopia (Ramadan and Morsel, 2003a, 2003b; Dagne and Johnsson, 1997; Dutta et al., 1994) and it appears that little or no work is done to improve the oil content in niger seed, at least not to the knowledge of the present author. Various authors reported varied oil contents in niger seed (Ramadan and Morsel, 2003a, 2003b; Dagne and Johnsson, 1997; Dutta et al., 1994). This variation in the oil content of the Ethiopian niger is an encouraging reality as the presence of variability by itself is indicative of the prospect to improving the oil content of the crop.

2 Objectives of the study

The genus *Guizotia* becomes important as one of the species, *G. abyssinica* is an important oil crop. Studying the genetic diversity of the *Guizotia* species and in particular of *G. abyssinica* lays the foundation for future work on the improvement of the crop plant. As all the taxa within the genus are related, some qualities of agronomic importance can be transferred from the wild or weedy species to the cultivated species through biotechnological manipulation. The study on oil quality is indeed crucial as it aids in the understanding of the diversity with regards to the oil content and oil quality pertinent to different populations of niger in Ethiopia. The present study was therefore, undertaken in an attempt to achieve the following objectives:

- To reveal the molecular genetic diversity among and within the different populations of niger growing in Ethiopia using inter simple sequence repeat markers.

- To elucidate the phenotypic variation with regards to the agronomic performance of niger populations collected from different regions in Ethiopia.

- To assess the genetic diversity and relationships among the wild *Guizotia* species using inter simple sequence repeat markers.

- To study the fatty acid profile of niger in an attempt to develop varieties that are true breeding for a rare fatty acid or a common fatty acid in unique proportions in the oil.

- To increase the content of oleic acid in niger seed oil by plant breeding.

3 Materials and Methods

3.1 The plant material

The plant materials were collected from different regions in Ethiopia. For *G. abyssinica*, plants on a farmer's field are regarded as a single population while for the wild *Guizotia* species, plants growing in the same locality are considered to belong to the same population. Seeds from individual plants were sampled separately in all instances. *G. abyssinica* was collected twice during the course of the study in 2003 and 2005. Because of uneven distribution, some taxa like *G. abyssinica* and *G. scabra* ssp. *schimperi* are collected from wider area because they have a wide distribution in the country, while others like *G. arborescens* and *G. zavattarii* are collected only from one region as they have a very restricted distribution in the country. Each taxon used in the study along with the region of collection is presented in table 1.

Table 1. The Guizotia materials used in the study along with the regions of collection in Ethiopia. (The full name of each region is given on paper II)

Axa	Regions of origin											
	Ha	Wo	Gn	Gj	Sh	Ji	We	Ar	Ba	Il	Si	Ka
G. abyssinica	X	X	X	X	X	X	X	X				
G. scabra ssp. *schimperi*	X	X	X	X	X	X	X	X	X	X	X	
G. scabra ssp. *scabra*						X	X			X		
G. villosa			X	X								
G. zavattarii											X	
G. arborescens												X

3.2 ISSR Diversity

3.2.1 DNA extraction and amplification

Seeds (achenes) that were brought from Ethiopia were planted in the green house in Sweden at SLU, Alnarp in the autumn of 2004. DNA was extracted from young leaves using the CTAB (cetyl-trimethyl ammonium bromide) method as applied by Assefa et al (2003b). For amplification by Polymerase Chain Reaction (PCR), fifteen primers were tested on a sample set and only five that produced consistently distinct bands with sufficient degree of polymorphism across the samples were selected. Amplification of DNA was carried out in a GENE AMP PCR thermocycler (HITACHI Ltd, Tokyo, Japan). The detail of the temperature profile for the amplification is explained in paper I&II. PCR products were electrophoresed in poly acrylamide gels from Amersham Pharmacia Biotech AB along with two lanes of size markers and visualized by silver staining on Hoefer automated gel stainer (Pharmacia Biotech). The bands were recorded as present (1) or absent (0).

3.3 Field trial

Niger plants were grown at two experimental sites in Ethiopia. They were planted at Haramaya University, east Hararghe (9° 24' N, 42° 2' E) on July 5 and Hirna experimental station in west Hararghe (9° 13' N, 41° 6' E) on July 7, 2005. Simple lattice design with two replications was used. In each of the two sites 2x4m plots consisting of ten rows with ten plants in each row was used. The spacing between the rows and between the individual plants in a row was 40cm and 20cm respectively. The descriptors used to characterize the plants for the traits studied were kindly provided by Dr. Adugna Wakjira from the Holeta Research Center, EARO. These quantitative traits were presented in paper III. Ten sample plants were selected from each plot for which all the data were recorded. Statistical treatment of the data was based on the means of the ten sample plants except for three variables, days to flower initiation, days to 50% flowering and yield per plot that were done on plot basis.

3.4 Fatty acid profile

For the analysis of the fatty acid profile, seeds were collected from individual plants from four regions of the country. However, only nine seeds showing relatively high oleic acid levels were used for subsequent breeding.

Seeds were screened for their oleic acid content after soaking in water overnight to facilitate the removal of the husks from the seeds for easy cutting of the cotyledons. Half of the cotyledons were removed from the seeds for fatty acid analysis. The details of the methodology used for the oil extraction from niger seeds are dealt with in paper IV. After the analysis of the fatty acid profile of the half seed, the remaining half seed is planted in the Biotron at SLU, Alnarp depending on whether the analysis shows elevated content of oleic acid. The starting materials consisting of nine individual seeds/plants are indicated in paper IV. The plants were cross pollinated with each other in the Biotron and harvested after they attained full maturity. Screening for the high oleic acid trait ensued by the half seed technique and the half seed expressing an exceptionally high oleic trait planted in the Biotron for a second round of breeding. The screening and breeding procedure continued for three generations after which niger plants breeding true for the high oleic trait were obtained.

3.5 Data Analysis

The 0, 1 data matrix recorded for the ISSR analysis were subjected to analysis by POPGENE software 1.3 (Yeh et al., 1999) which generated single population as well as group population gene frequencies for the *G. abyssinica* populations as well as for the wild *Guizotia* populations. The resulting gene frequencies were used to construct the Unweighted Pair Group Method using the arithmetic average (UPGMA) dendrogram by another software package, Genetic Distances and Phylogenetic Analysis (DISPAN, 1993). The Nei (1972) genetic distances matrix between the populations was generated by the

POPGENE software (Yeh et al., 1999) and the Nei (1973) genetic diversity parameters were analyzed using the DISPAN (1993) software. The mean Shannon-Weaver diversity indices were calculated following the procedure used by Assefa et al. (2002).

For the analysis of the phenotypic characters NTSYS-pc software package (Rohlf, 2000) and the JMP statistical software (SAS Institute, 2004) were used. The NTSYS was used to construct the UPGMA for the accessions as well as for the regions of origin using the plot means and the regional means of each character respectively. The plot means were used for the principal components analysis for the accessions grown at the two experimental stations. Principal components analysis was also performed using the regional means of each trait and eigenvalues and eigenvectors calculated from the components axes using the correlation matrix produced for each character. The values for the eigenvectors from the first four principal components axes for Haramaya and Hirna experimental sites is presented in paper IV.

Separation of the fatty acid methyl esters was done by gas chromatography (Shimadzu GC-17A, Kyoto, Japan) with a flame ionization detector (FID). A WCOT fused silica capillary column (50mx0.32mm), CP-wax 58 (FFAP)-CB (Chromopack, Middleburg, The Netherlands) was used. The details of the temperature profile are dealt with in the paper.

4 Results and Discussion

4.1 Genetic Diversity of the *Guizotia* species (papers I & II)

The five primers used for this study were summarized in table 2 of paper I&II along with the total markers generated by each primer and the number of polymorphic loci for each. The primers amplified a total of 118 and 145 scorable bands for *G. abyssinica* and the wild *Guizotia* species respectively. Of the five primers employed, primer UBC 841 produced more bands across all the wild *Guizotia* populations studied with 33 bands of which 32 were polymorphic followed by primer UBC 834 with 31 total bands out of which 29 were polymorphic. As both these primers consist of dinucleotide repeats, the finding suggests that dinucleotide repeat primers produce more bands in the wild *Guizotia* species. This is in conformity with Yao et al. (2008) who suggested that the dinucleotide repeat motifs are more frequent than the tri or tetranucleotide repeats. The same principle holds for *G. abyssinica* also, except that it is another dinucleotide repeat UBC 888 that produced the most bands. The least number of bands was recorded for a tetranucleotide repeat primer UBC 878 for the wild *Guizotia* species and a trinucleotide repeat primer UBC 866 for niger. The fact that Primer UBC 841 with its dinucleotide GA repeats produced the most bands suggests the abundance of GA repeat motifs in the genome of the wild *Guizotia* species. The highest level of polymorphism was scored for *G. abyssinica* followed by *G. scabra* ssp. *schimperi* with 89.83% and 88% polymorphic loci respectively. *G. arborescens* was found to be the least polymorphic among the *Guizotia* species exhibiting only 68.2% polymorphic loci.

It was observed that the coefficient of genetic differentiation (Gst) was the least (0.0918) for *G. abyssinica* and the highest (0.3610) for *G. scabra* ssp. *schimperi*. *G. scabra* ssp. *scabra*, *G. villosa* and *G. zavattarii* had coefficients of genetic differentiation of 0.2552, 0.2551 and 0.0985 respectively. Likewise, the total genetic diversity (Ht) was found to be highest (0.4115) for *G. abyssinica* of which 90.8% was explained by the genetic diversity occurring within populations. Among the wild *Guizotia* species, the total genetic diversity ranged from 0.1791 for *G. zavattarii* to 0.2401 for *G. scabra* ssp *schimperi* with *G. villosa*, and *G.*

scabra ssp *scabra* exhibiting total genetic diversity of 0.1867 and 0.2117 respectively. In all the *Guizotia* taxa investigated, the within population genetic diversity (Hs) was found to be higher than the among population genetic diversity (Dst). The within population genetic diversity for all the *Guizotia* taxa ranged from 0.1391 for *G. villosa* to 0.3738 for *G. abyssinica* with *G. scabra* ssp. *schimperi*, *G. scabra* ssp. *scabra* and *G. zavattarii* possessing Hs of 0.1534, 0.1576 and 0.1615 respectively. In fact *G. abyssinica* had one of the lowest among population genetic diversity and the highest within population genetic diversity among the *Guizotia* species. Thus, as it is generally true for all out crossing species, most of the genetic diversity of the *Guizotia* species is accounted for by the variability among the individuals of a population than among the populations. The small Gst value amounting to 0.0918 for *G. abyssinica* is also an evidence of the greater within population variation than among them. The small Gst value for *G. abyssinica* indicates that there is only a small genetic differentiation among the niger populations growing in Ethiopia. This could be assumed to be the result of the high degree of out crossing resulting in a relatively high gene flow among the different populations of the country.

4.1.1 Genetic Distance and Gene flow

There seems to be a strong correlation between the genetic distances and the geographic distances between the populations of the taxa. The standard genetic distance and the Nei (1972) genetic distance was found to be least between populations from a region than between populations from different regions. Thus for *G. abyssinica*, the highest genetic distance (0.3261) was between populations of Tiyyo and Koladi from Jimma and Wollo regions respectively, while the lowest distance was between Kombolcha and Gerado both from Wollo region. The standard genetic distance for the regions' populations of *G. abyssinica* ranged from 0.0281 between populations of Wollo and Gojam to 0.1148 between niger populations of Hararghe and Jimma. Among the wild *Guizotia*, the standard genetic distance ranged from 0.1188 (between *G. scabra* ssp. *schimperi* and *G. villosa*) to 0.2740 (between *G. scabra* ssp. *schimperi* and *G. arborescens*). Thus, the genetic distance between populations reflects more or less the level of similarity and the degree of relatedness of populations. In terms of species relatedness, the ISSR analysis results indicate that *G. scabra* ssp. *schimperi* is more closely related to *G. villosa* with a genetic distance of 0.1188 between them than to any other wild *Guizotia* taxa. Similarly *G. zavattarii* is more closely related to *G. scabra*

ssp *scabra* and *G. arborescens* with a genetic distance of 0.1819 and 0.1716 from *G. scabra* ssp *scabra* and *G. arborescens* respectively.

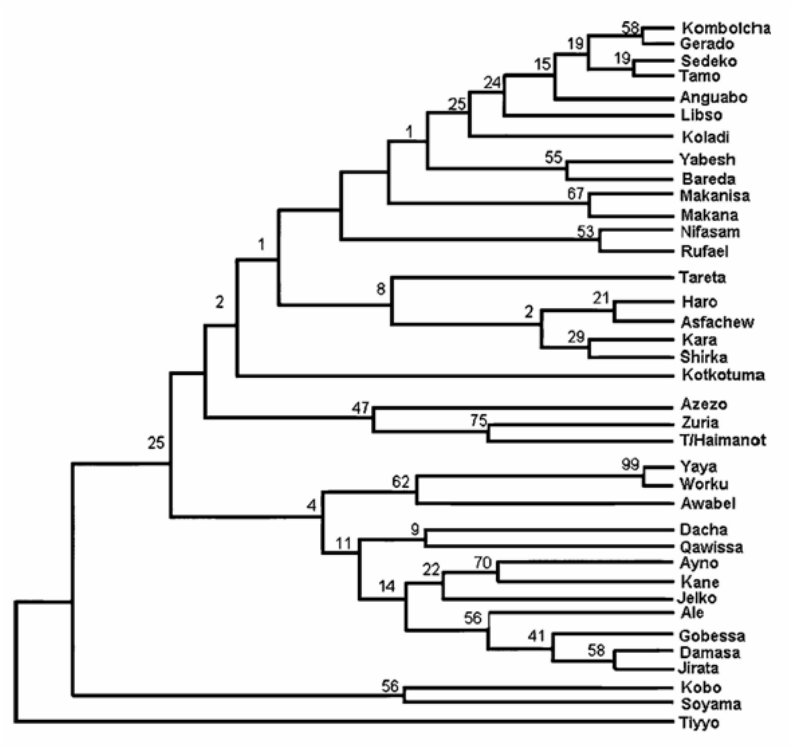


Figure 1. UPGMA clustering pattern of 37 populations of *Guizotia abyssinica*.

The mean Shannon-Weaver diversity index for *G. abyssinica* data was 0.8841 while the indices for the wild *Guizotia* species ranged from 0.5791 (*G. arborescens*) to 0.7373 (*G. scabra* ssp. *schimperi*). The average heterozygosity of the wild *Guizotia* species ranged from 0.1867 for *G. villosa* to 0.2410 for *G. scabra* ssp. *schimperi*. The high genetic diversity exhibited by the *Guizotia* taxa can be ascribed to the out crossing mode of their reproduction and the greater capability for the dispersal of their pollen. The *Guizotia* are pollinated by insects mainly by bees. As indicated by Xiao and Gong (2006), it is mainly the ability for pollen dispersal that leads to higher gene flow.

The amount of gene flow estimated as Nm = 0.5(1-Gst)/Gst was found to be (0.23716) for *G. abyssinica*. Among the wild taxa, it ranged from 0.8849 for *G. scabra* ssp. *schimperi* to 4.5760 for *G. zavattarii* with *G.*

scabra ssp. *schimperi* and *G. scabra* ssp *scabra* showing Nm values of 1.5473 and 1.6782 respectively. As the Nm is indicative of the number of migrants per generation (Jian et al. 2004), it indicates that *G. abyssinica* has a high gene flow in comparison to most of the other *Guizotia* species though there is some variation with regards to the amount of gene flow among the *G. abyssinica* populations of the different regions. Among the different populations of *Guizotia abyssinica* grown in the different regions in Ethiopia, it was observed that the niger populations from Wollo had the highest gene flow with an Nm of 3.8439 and those from Gojam had the least Nm of 1.9461. On the other hand all the wild *Guizotia* taxa had Nm values lower than that of *G. abyssinica* except for *G. zavattarii*. The lowest Nm (0.8849) was recorded for *G. scabra* ssp *schimperi* and the highest Nm (4.576) for *G. zavattarii*. As Nm is a measure of gene flow, and therefore, migration of individuals/pollen, it follows that the Nm value for a given species is affected by the geographic distance between populations as well as human interference. The low Nm value for *G. scabra* ssp. *schimperi* is a reflection of the countrywide distribution of this taxon and therefore the extent of the geographical distance separating individual populations of the taxon. Likewise, among the populations of *G. abyssinica*, the low Nm for the populations of Gojam indicates that the sampling areas in Gojam are indeed far apart compared to the collection sites of the niger populations of Wollo.

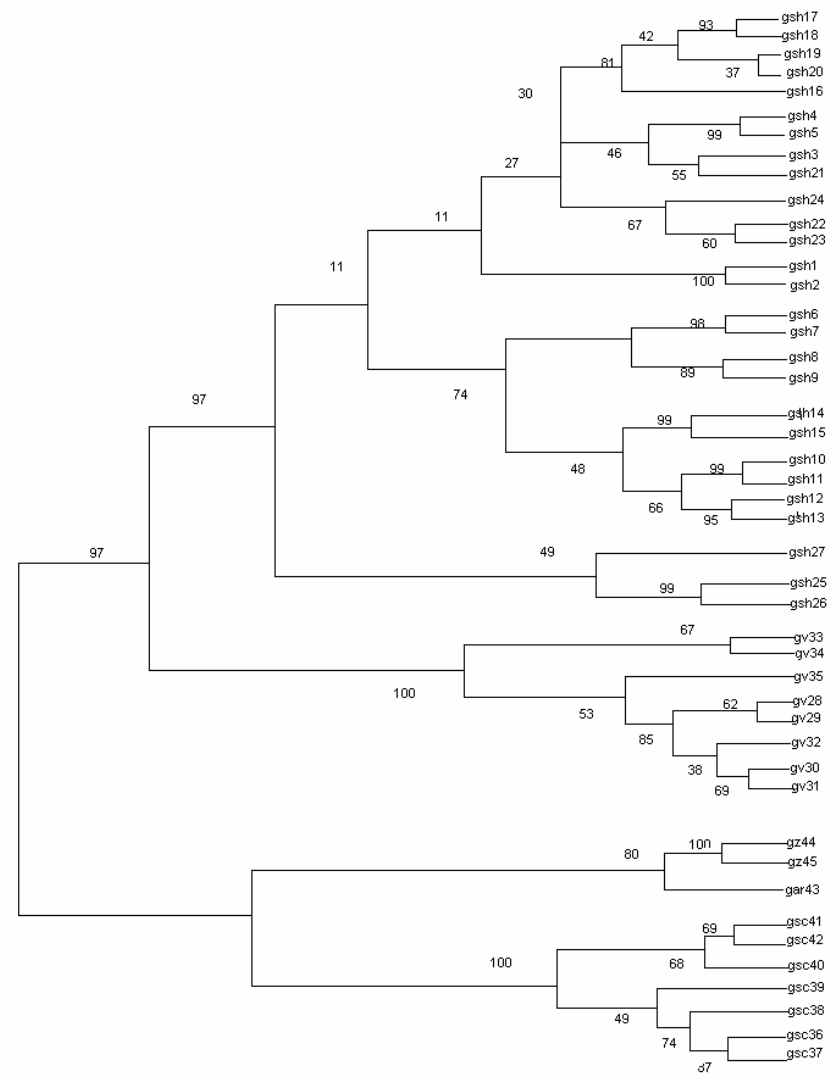


Figure 2. Clustering pattern of 45 populations of wild *Guizotia* generated by UPGMA cluster analysis

While the ISSR technique was able to clearly discriminate among the various wild *Guizotia* taxa from Ethiopia, it did not, however, reveal clear cut identification when it comes to the niger populations from the different niger growing areas in Ethiopia. As observed, the interspecific standard genetic distance of the wild *Guizotia* taxa ranged from 0.1716 to 0.2740 compared to the inter-regional standard genetic distance for niger populations which only ranges from 0.0281 to 0.1148. As a result,

the UPGMA dendrogram based on the standard genetic distances was not able to discriminate among the niger populations growing in different area of the different regions in the country. The clustering pattern indicated on figure 1, seems to have been influenced more by the type of niger grown in the regions than by ecogeographical factors. This is evidenced from the dendrogram where the populations were grouped into two main clusters. The first cluster contains mainly the populations from Wollo and Hararghe and the second cluster containing the populations from the rest of the regions. It is known that farmers in Wollo and Hararghe grow the early maturing 'Bunigne' niger in contrast to those in Wellega, Shewa, Gojam and Jimma who mainly grow the late maturing 'Abat' niger (Getinet and Sharma, 1996). It is highly probable that the clustering pattern of the niger populations reflect both the varietal as well as the ecological factors. This is further confirmed from the UPGMA dendrogram produced for the regions of origin where two major clusters one containing the eastern and the northern regions and the other containing the rest of the regions is produced.

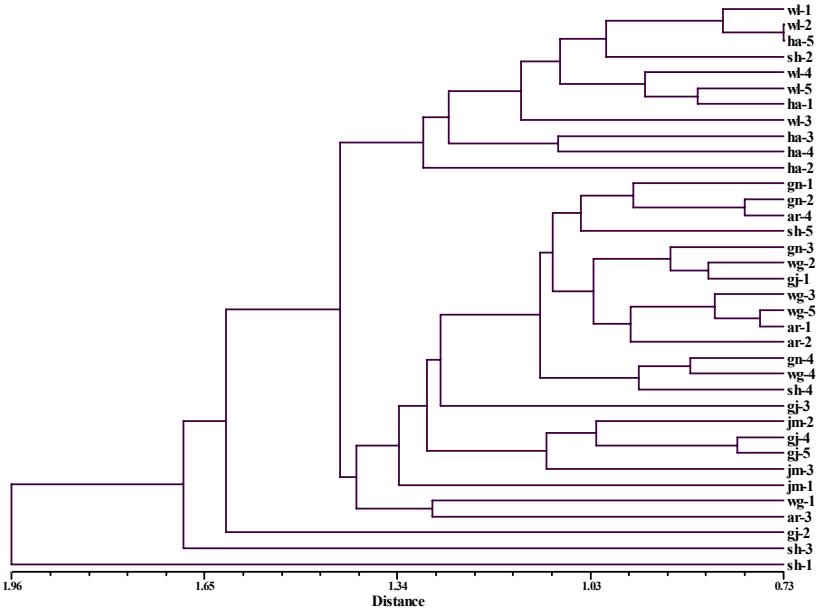
Among the wild *Guizotia* species the interspecific genetic distance was sufficiently large to group them into distinctly separate clusters on the UPGMA dendrogram (Figure 2). Based on the genetic distances and the clustering pattern obtained, it is the authors' opinion that *G. scabra* ssp *schimperi* be treated as a separate species, because, it is in fact more closely related to *G. villosa* than to *G. scabra* ssp. *scabra*. This opinion is shared by other authors as well whose argument is based on molecular as well as cytogenetic evidences (Geleta et al., 2007a; Murthy et al., 1993, 1995).

4.2 Phenotypic Diversity of niger (paper III)

Thirty six accessions that were previously used for genetic diversity studies using ISSR were also used for the field evaluation of agronomic characters of niger. The results indicate wide variation among the different accessions with regards to several of the quantitative characters investigated. The variations concern traits of agronomic importance such as days to flower initiation, days to 50% flowering, seed size,

number of flower heads per plant, number of seeds per head, yield per plant, yield per plot, plant height and the extent of branching.

It was observed that the thirty six accessions originating from the eight niger growing regions of the country fall into two main groups, the variability being contributed mainly by days to flower initiation, days to 50% flowering, plant height, number heads per plant, number primary branches and yield per plot. The results confirmed the notion that there are two main types of niger growing in Ethiopia that greatly differ in the duration to maturity (Almaw and Teklewold, 1995; Getinet and Sharma, 1996). The present study revealed that the niger populations originating from Hararghe and Wollo have many agronomic characteristics in common that are in stark contrast to those from the other regions of the country. Notable among these characters contributing to the observed variations among the accessions are primarily plant height, days to flower initiation, days to 50% flowering, number of heads per plant and 1000 seed weight.



Figur 3. UPGMA Clustering Pattern of the niger populations used in the study

Inter-regional variations among the accessions were based on the regional means for each character. Accordingly, the first four principal components accounted for about 91% of the total variation among the

regions populations. Eigenvectors from the first, second, third and fourth principal components axes accounted for about 43, 25, 16 and 7% of the total variation respectively. The most important variables of the first principal components axis were days to flower initiation, days to 50% flowering, number of heads per plant, plant height, yield per plot and 1000 seed weight, while in the second principal components axis, number of primary branches, number of secondary branches, number of heads per plant and 1000 seed weight were the most important variables responsible for the variations observed among the accessions.

Days to flower initiation, days to 50% flowering, plant height and the number of primary branches contributed most to the variations observed in the first principal components axis for the niger populations grown at Haramaya university while number of heads per plant, number of primary and secondary branches, yield per plant and 1000 seed weight happened to be the most important variables of the first principal components axis for the populations grown at Hirna experimental site.

These characters affect the time taken for the completion of the plant's life cycle as well as the productivity in terms of seed yield. Thus, all the accessions from Wollo and Hararghe are of the early maturing type while most of the accessions from the other regions, especially those from Gojam, Wellega and Jimma are of the late maturing type. Thus, the Ethiopian niger germplasm consists mainly of these two types (Almaw and Teklewold, 1995), though there is a third type of niger that is frost resistant (Getinet and Sharma, 1996). The 'Bunigne' niger grown in Wollo, Hararghe and north east Shewa are early maturing small plants producing more flower heads per individual plants, and with relatively smaller seeds, while the 'Abat' types are late maturing tall plants with fewer flower heads and producing relatively larger seeds compared to the early maturing types. There is significant positive correlation of plant height to the duration to 50% flowering. Thus the earliest 'Bunigne' accession (sh-1) took 63 days to flower while the latest 'Abat' accession (gj-2) took 109 days to flower. The accessions from Wollo and Hararghe had average heights of 130.3cm and 133.8cm respectively while the 'Abat' accessions from Gojam and Wellega were found to be taller on the average even than the other 'Abat' accessions from the rest of the regions measuring 146.7cm and 150.8cm respectively.

Based on the principal components analysis, scatter plots were produced for the accessions obtained from the different regions of the country. The accessions form two separate clusters, the Wollo and Hararghe

accessions being grouped together. The UPGMA dendrogram for the accessions (Figure 3) also depicted a similar pattern producing two main clusters, one of which mainly consists of the accessions from Wollo and Hararghe while the second cluster consists of the accessions from the other regions. Likewise, the UPGMA dendrogram based on the regional means for each character produced two main clusters placing Wollo and Hararghe together in a separate cluster. The second major cluster for the regions consists of two distinct sub clusters where Gonder and Shewa were grouped together in a sub cluster and Gojam, Wellega and Arsi likewise placed in the second sub cluster. Jimma, however, remained solitary in the second sub cluster connected only distantly to the two sub clusters of the second major cluster. This indicates that the niger grown in Jimma, though of the 'Abat' type is , however, sufficiently different from the other 'Abat' accessions grown in the other regions. It has been observed from table 2 (paper III) that some of the unique features of the Jimma populations separating them from the other accessions might have been the extent of primary and secondary branching and the number of seeds per head. The average number of primary and secondary branches of the accessions from Jimma exceeds the number recorded for the other accessions studied. Thus, based on the study, the Jimma populations of niger could be described as late maturing plants that are highly branching and producing fewer seeds per head.

Niger populations growing in Ethiopia harbour rich genetic diversity. Previous investigations using molecular markers have shown that great variation is indicated within the same population growing on a field as well as among different populations growing in different regions of the country (Geleta et al., 2007b, 2008; Petros et al., 2007). The variations existing among the niger populations in the different regions of the country are to a large extent attributed to the inherent variability in important agronomic characters existing between the different strains of niger, whether early maturing or late maturing, and to a lesser extent to ecogeographic factors unique to the various regions of origin. The present study has indicated these important characters differentiating between these two niger strains. These important agronomic traits inherent in the two strains and responsible for producing such striking contrast between them relate to the number of days to flower initiation and 50% flowering both of which determine the duration to maturity. Equally very important traits marking the phenotypic variation between the two types of niger are the number of flower heads per plant, the extent of branching and seed size. As only very little work is done so far to improve the oil and seed yield of niger, it is believed that the future

holds encouraging prospects in this regard for the improvement of important agronomic qualities of this crop. The ideal niger plant needs to be early maturing with short and sturdy stems, for which candidates could be sought among the 'Bunigne' types. It also needs to have larger seed size producing greater yield in terms of oil and seed for which the 'Abat' types could serve as good starting materials.

4.3 Comparing the molecular and phenotypic diversity of niger (paper I & III)

The phenotypic characterization of niger for agronomic traits and the molecular diversity analysis using ISSR markers produced results that are more or less in harmony, though not totally congruent. Two major clusters with smaller sub clusters are produced in both the molecular analysis and the field evaluation. In both cases, the accessions from Wollo and Hararghe were included in the first major cluster. In fact the first major cluster produced for the phenotypic characters of niger wholly consisted of the accessions from these two regions except for one accession from Shewa (sh-2) which had paired with sh-3 in the ISSR data. It can be observed that the same accessions grouped in the same cluster in the ISSR analysis were placed in two separate clusters in the dendrogram for the field data. Some accessions occurring in the first sub cluster of the second major cluster of the field data were grouped in the first cluster of the ISSR data. The first major cluster of the ISSR data consisted of nineteen accessions all of which occur in the two clusters of the field data except for two populations each from Arsi and Gojam. Conversely, there are twenty one accessions included in the first two clusters of the field data. Two accessions each from Arsi and Wellega and an accession each from Shewa and Gonder were not included in the first major cluster of the UPGMA dendrogram produced for the ISSR data. An accession from north-east Shewa (sh-1) which was the earliest to flower both at the Haramaya and Hirna field experimental sites was grouped together with the Wollo and Hararghe accessions in the dendrogram produced for the ISSR data but remained an outlier in the dendrogram for the field trial. The duration to flowering as well as other important agronomic parameters suggest that this accession indeed belongs with the Wollo and Hararghe accessions. None of the Jimma accessions were grouped in the cluster containing the Wollo and Hararghe accessions in both the dendrograms produced for the ISSR

and the field data. Based on the phenotypic trait evaluation of the field data, they were grouped together with the accessions from Gojam. This clustering pattern of the Jimma accessions along with the accessions from Gojam is convincing when one considers that the accessions from these two regions have a number of agronomic features in common notable among which is the duration to flowering. Taking the regions of origin of the materials in retrospect, the molecular analysis and the phenotypic evaluation of agronomic characters more or less agree. The UPGMA dendrograms for the regions in both analyses produced two major clusters. Jimma was an outlier in the dendrogram for the ISSR data and was not grouped with any of the regions in the field data. Wellega was grouped with Gojam in the analysis of the field data but with Arsi based on the ISSR data. All these three regions, Gojam, Wellega and Arsi are known to grow the late maturing types of niger.

The dendrograms for the field and ISSR data are produced using the Euclidean and the standard genetic distances respectively. The variation in the pattern of clustering in the two data analyses might have been the result of these two different methods of analysis as well as the different data generated from these two completely isolated works. What can be asserted from these two analyses results is that the field evaluation of the phenotypic characteristics of niger and the genetic analysis using ISSR markers revealed slightly different patterns for the diversity of niger populations growing in Ethiopia.

As generally, the revelation of molecular diversity is manifested in phenotypic expression, there can be a host of traits for which one accession may differ from the other. Thus, all the accessions used for the present study do not group together solely on the basis of the region of origin. This indicates that, while eco-geographic feature of their respective regions is a factor, there seems to be other factors, namely, material transfer between neighbouring regions or difference in the type of strains grown in different regions that play roles in assigning the accessions to the different clusters. It is also evident that the late maturing types of niger are more varied compared to the early maturing ones.

4.4 Breeding for high oleic acid in niger (paper IV)

The oleic acid content in the nine materials selected for breeding ranged from 17-30.6%. This was increased to about 35.2% on the average after the first round of breeding. It became clear, however, that the oleic acid content from a single cross and indeed even from the same flower head were not uniform in the percent oleic acid content but showed great variation. Although the difference in the oleic acid content between the lowest and the highest in the starting material was only about 14%, this variation, however, increased in the progeny seeds to about 79% after the first harvest. Whereas no seed used as the starting material for the breeding experiment had oleic acid content above 31%, it was observed that after the first round of breeding a number of progeny seeds had percent oleic acid higher than 50% with one seed's oleic content equalling 83%. The increase in the oleic acid content of progeny seeds continued producing more than 20% of the seeds with oleic acid content of greater than 80% after the second round of breeding with an average of about 53.2%. Even then, there was great variation in the percent oleic acid content among the individual seeds. Only those seeds with oleic acid content of more than 80% were selected for the third round of breeding, after which almost all progeny seeds had oleic acid content of 80% or more

The study showed the possibility of increasing the oleic acid content in niger seed oil by repeated selection and breeding. In the present study it was made possible to increase the oleic acid in niger seed oil from approximately 8.4% in the ordinary niger materials from Ethiopia to over 80% after selection and breeding in a controlled environment at Alnarp (Fig. 4). It was also observed that plants whose oleic acid content is approximately $\geq$ 79% are true breeding for the high oleic trait

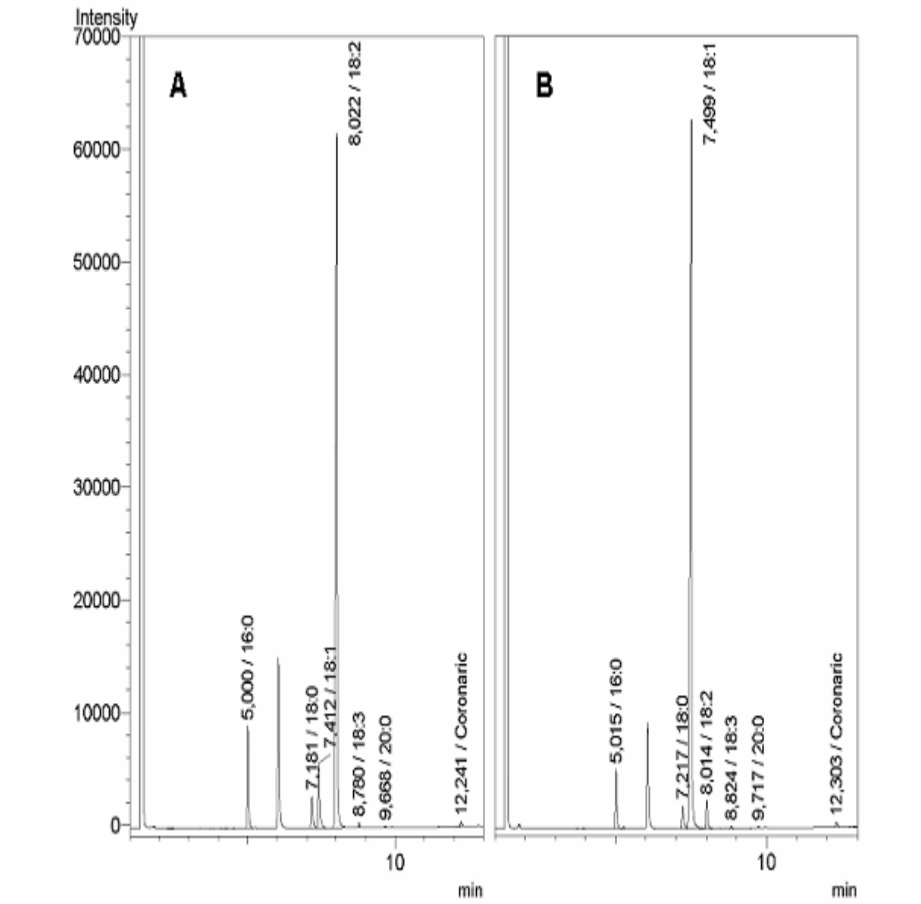


Figure 4. Fatty acid profile of niger. A. ordinary niger material. B. high oleic acid yielding niger.

The mode of inheritance of the high oleic trait in niger or the number of alleles involved in determining the oleic/linoleic ratio is not yet known. It is reported that in the sunflower mutant 'Pervenets' the interaction of three alleles control the oleic acid content in the seeds (Martinez et al 1989). Schuppert et al (2006) found out that the increase in the sunflower variety Pervenets is partly caused by a mutant allele 'Ol' exerting incomplete dominance. Thus it is possible that more than one allele may be involved in the production of the high oleic acid content in niger. The number of genes and alleles involved in the expression of the high oleic trait in niger is presently the subject of investigation at SLU, Alnarp.

The changes in the biosynthetic pathway resulting in the accumulation of oleic acid in niger seed also needs to be investigated. As the biosynthetic pathway for the production of oleic and linoleic acids entail series of desaturation steps leading from 18:0 to 18:1 and from 18:1 to 18:2, it is envisaged that the phenomenon leading to the accumulation of oleic acid in niger may be due to either of the following reasons. 1. increased activity of 9 desaturase enzyme, the result of which is increased production of oleic acid from stearic acid (18:1). 2. Reduction or loss of activity of the 12 desaturase enzyme that utilizes oleic acid in the production of linoleic acid. The net effect of these activities is the preponderance of triolein as the predominant triacylglycerol (TAG) molecular species.

5 Concluding remarks

Niger populations growing in Ethiopia harbour rich genetic diversity. There is great variation among the populations growing in different regions and localities as well as among individuals of the same population. This variability could be made into good use by breeding for niger plants with the desired agronomic characters. The niger ideotype should be an early maturing short plant with strong stems whose flowering and maturity are synchronized. It is imperative that the plant produces superior yield in terms of seed or oil.

The early maturing and late maturing niger plants differ with regards to several traits such as the span of their life cycle, height and seed size. The late maturing niger types are also known to have higher oil content and seed yield than the early maturing ones. In fact, there is great variation even among the late maturing niger types themselves with respect to many characters of agronomic importance. Future attempts to improve the seed or oil yield of niger need to exploit this diversity to develop the ideal niger variety with the desired agronomic qualities that are well suited to the environmental conditions of the areas of cultivation.

Niger strains with exceptionally high oleic acid have been developed in the present study. There could as well be a host of other valuable traits waiting yet to be explored as the plant is very little studied. Future research trend in this regard should aim at the development of hybrid varieties that are high yielding both in terms of oil content and oleic acid content.

High genetic diversity was observed in the wild *Guizotia* species both within and among the populations of the respective species. As reported by several workers, niger shares more similarity with *G. scabra ssp. schimperi* and *G. villosa* than with the other members of the taxa. As these taxa are known to form inter-specific hybrids with relative ease, there is a good likelihood of transferring important agronomic qualities from the wild species to the cultivated one.

Some of the *Guizotia* species such as *G. scabra ssp. schimperi* are widely distributed in the country and are not facing any threat for their survival, at least not in the immediate future. Others like *G. arborescens* on the other hand, are limited to a small area in the country and are at a greater

risk due to human activities in the area, hence the urgent need for conservation and preservation of the germplasm.

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Acknowledgements

My heart-felt gratitude to the following persons who were very concerned about my work and deeply involved with me.

Professor Arnulf Merker, my main supervisor for his guidance and advice throughout the course of my studies, for his kindness and responsibility in all of my works. I owe him so much for his concern about my wellbeing as well as for his vital contribution to all the achievements in my studies.

Doctor Anders, S. Carlsson, my co-supervisor from whom I have learned a lot about laboratory analyses of lipids and who has always been available whenever I needed help. His gentle and kind advice and above all his critical suggestions were indispensable for the successful completion of my works on fatty acids.

Professor Sten Symne who felt it his responsibility to help me finish my experiment on oleic acid with success. Who was deeply involved in the work and highly enthusiastic about the outcome, whose advice, critical suggestions and constant encouragement proved absolutely essential for the successful completion of my last experiment.

Doctor Habtamu Zeleke, my Ethiopian Supervisor for his help when I conducted the field trial, for his concern and encouragement.

I am very grateful to Ann-Sofie Fält who showed me DNA extraction and gel electrophoresis and who crossed the plants throughout the winter and autumn of 2008 while I was away.

I would like to express my deepest gratitude to Anders Smolka who helped me in from the cold when I broke my leg in 2007 and to Åsa Ekman who gladly provides help with the GC on every occasion I needed.

I would also like to express my heart-felt gratitude to Doctor Mulatu Geleta and Monica Lotfinia, who have been of great help during the times I was a patient because of a broken leg. I used to obtain their unreserved help whenever I needed it, for which I am very grateful.

My deepest gratitude goes to Professor Waheeb Heneen and Iris Heneen for their kind hospitality and the joyous and relaxing times we had together.

I am greatly indebted to Faris Hailu who helped me in the analysis of my results. I greatly appreciate his sincerety, patience and profound knowledge.

I would like to extend my gratitude to Professor Olof Hellgren and Doctor Göran Nilsson who helped me when I raised my plants in the Biotron. I am also highly indebted to Göran Olsson and Karl-Johan Bergstrand for their help in the greenhouse.

I am very thankful to Helen Lindgren, Susanne Hjerdin, Annelie Ahlman, Marissa Prieto-Linde, Jonas Hansson and Britt Green who gladly offered their help whenever I needed.

I am especially grateful to all the people I have known here in the department of Plant Breeding and Biotechnology. I would like to extend my sincere thanks to the good time I had with you all.

I would like to express my gratitude to the Swedish International Development Agency, Sida-SAREC for financing this project, and to Haramaya University for giving me the opportunity to study in Sweden. I am particularly grateful to Professor Belay Kassa, Doctor Asha Yahya, And Doctor Chemeda Fininsa.

I would like to thank Doctor Adugna Wakjira and Doctor Ketema Belete for providing materials and advice.

I would like to express my thanks to the former Ethiopian PhD students in Sweden, Sissay Menkir, Esayas Aga, Jemma Haji, Hussein Hamda, Genet Birmeta, Mengistu Urge, Tiliye Feyissa, Yoseph Mekasha &Yitbarek W/H.

I am very grateful to Wondossen Bedilu, Abdulfetah Abdulahi, Yemane Aklil and Haimanot Bizuneh who helped me in the collection of the plant material and to Melaku Zewde who managed the plants during the field trial.

Finally I would like to thank my relatives Dr. Bisrat Mikael, Theodros Mikael, Dire Mishel, Eyasu Mishel, Maria Petros, Tony Petros, Lule Mikael and my aunts Martha Rufael and Rosa Poli.